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Assessment of rice genotypes to susceptibility of sheath blight disease caused by *Rhizoctonia solani* AG1-IA

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ABSTRACT

Rice crop endures several biotic stresses among which sheath blight is one of the devastating diseases. This disease is caused by necrotrophic fungus *Rhizoctonia solani* AG1 IA that reduces 20 to 40% yield. Forty two diverse rice genotypes were evaluated against sheath blight under artificial epiphytotic condition in the field of National Wheat Research Program Bhairahawa, Nepal during the year 2019. Four disease variables *viz.* PDLI (Percent diseased leaf incidence), PDTI (Percent diseased tiller incidence), PRCHI (Percent relative collar height infection), and AUDPC (Area under disease progress curve) were considered for evaluation of genotypes. Out of forty two genotypes Sabitri, GSR 310 and Hardinath-3 were found moderately resistant with mean AUDPC values 217.99, 252.78 and 214.67 per day respectively. Furthermore IR 15D 110, Pant-1, NR 2152-23-1-2-1-1-1-1 and IR 82635-B-B-114-3 were found moderately susceptible with mean AUDPC values 438.48, 445.55, 421.81 and 437.59 respectively. Moderately resistant genotypes *viz.* Sabitri, GSR 310 and Hardinath-3 had PDLI range 30.98-31.67, PDTI range 10.56-15 and PRCHI range 9.01-28.64 whereas moderately susceptible genotypes IR 15D 110, Pant-1, NR 2152-23-1-2-1-1-1-1 and IR 82635-B-B-114-3 had PDLI range 31.25-51.29, PDTI range 25.82-38.75 and PRCHI range 22.18-45.8. Disease variables PDLI, PDTI and PRCHI were positively and significantly correlated with AUDPC with correlation coefficient value 0.75, 0.65 and 0.62, respectively. Moderately resistant rice genotypes found in this study could be evaluated for yield potential and its stability across different geographical region of Nepal and could be a good alternative against sheath blight diseases for Nepalese farmers.

Keywords: Evaluation, rice genotype, sheath blight, *Rhizoctonia solani*, disease

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1 Introduction

Rice (*Oryza sativa* L. / *Oryza glaberrima*) is third most produced cereal with an estimated production around 500 million metric tons in 2019/20 (Shahbande, 2020); provide staple food to an estimated human population 3 billion throughout the world (Yadav and Kumar, 2019). It provides more than 50% of human diet calorie and abundant amount of protein to about 520 million people thriving poverty in Asia (Muthayya et al., 2014). More than 90% of world rice production is produced and consumed in Asia; China being largest rice producer contributing 212 million metric tons whereas India is second largest rice producer contributing 172 million metric tons (FAO, 2020). In Nepal two third population rely on agriculture for their income, employments and livelihood (NSCA,

2013). Rice is number one cereal and main staple food of Nepal cultivated in 1.48 million ha with an estimated production around 5.6 million metric ton (MoALD, 2019). Rice fulfill 50% of the total required calorie to 29.6 million Nepalese people and share 21% and 7% agricultural gross domestic product and GDP respectively (Basnet, 2017). Biotic and abiotic stresses and socio-economical influences are the major aspect playing pivotal role for rice production in Nepal.

Sheath blight of rice caused by a basidiomycetous necrotrophic fungus *Rhizoctonia solani* (Kuhn) [teleomorph-Thanatephorus cucumeris Frank (Donk)] is the second most prevalent disease next to blast of rice in the world (Molla et al., 2020). *Rhizoctonia solani* (Kuhn) infects a wide range of host plants belonging to 188 genera from 33 different families (Srinivasachary et al., 2010; Sattari et al., 2014). *Rhizoctonia solani* has high genetic variability making it capable of infecting wide host range, comprises 14 anastomosis groups *via*. AG1 to AG13 and 14th AGB1 is a bridging group (Carling et al., 2002). DNA sequence homology and morphology of sclerotia dissected AG1 group into three subgroups *viz*. AG1-IA, AG1-IB and AG1-IC (Sneh et al., 1991). It is widely appreciated that AG1-IA isolate is the causal organism of sheath blight of rice (Molla et al., 2020). The typical symptoms of sheath blight are water-soaked oval/ spherical/irregularly elongated, light grey to whitish lesion with dark margin on leaf sheath or on leaf blades (Molla et al., 2020). The propagules of this pathogen are either sclerotium or runner hyphae lead to initiation of disease by penetrating the host tissue with infection cushion or lobate appressoria or both (Marshall, 1980).

Rhizoctonia solani, causing sheath blight of rice aggravates in response to high temperature (28-32 °C), high nitrogen fertilizer doses, semi dwarf high yielding varieties (Savary, 1995), higher seeding rate or denser rice canopy, high humidity (85-100%) and prolonged duration of canopy wetness (Kannaiyan and Prasad, 1983).This disease initiate at around late tillering stage to stem elongation stage and circumvent at panicle differentiation stage. It has tendency to reduce yield on an average 20% to 42% under artificially inoculated field plots (Cu, 1996). Rice sheath blight impregnate throughout the rice growing areas of Nepal, established as one of the major threats to rice production (Manandhar et al., 1992). It can cause on an average 28% yield loss and infected grains breaks during milling process (NRRP, 2000).

Cultural practices, chemical control, biological control and resistant breeding are the means for plant disease management. Resistant breeding for managing plant diseases is most preferable technique because it is economical, environment friendly and easily adopted by farmers. Rice sheath blight disease is one of the most difficult diseases to manage because of wide genetic diversity of *R. solani*, extended host range, surviving ability of sclerotia from season to next season and its enduring nature to adverse condition (Molla et al., 2020). Using different breeding technique more than 50 QTLs have been identified using various mapping population (Lavale et al., 2018; xiang Zeng et al., 2015). Two reliable QTLs has been revealed from a recent genome wide association study (Chen et al., 2019), however, no QTLs has been characterized till to date (Singh et al., 2019). Evaluation of rice genotypes against this disease is ever lasting process to identify resistant rice genotypes. The objective of this research is to evaluate the advanced lines rice genotypes against the isolate of *R. solani* causing sheath blight of rice in the environmental condition of Nepal.

2 Materials and Methods

2.1 Genotypes

Forty two advanced lines rice genotypes were obtained from National Rice Research Program, Baniniya, Dhanusha, Nepal and evaluated during main season of rice in the year 2019. These genotypes were selected based on their diverse genetic background, cultivation areas, maturity time, morphological and quality traits. These materials were under different stage of selection *viz*. IET (Initial evaluation trail), CVT (Coordinated varietal trail), CFFT (Coordinated farmer's field trial) and PVS (Participatory variety selection). The experiment was conducted in 'D' Block of National Wheat Research Program, Bhairahawa, Nepal situated at 27°32′ N and 83°28′ E and 105 masl.

2.2 Experimental layout and cultural practices

The seed bed was prepared after harvesting of preceding wheat crop. Fifty cm raised dry seed bed was prepared with 1 m width and length as per requirement (IRRI website). One gram seeds of each genotype were sowed in a row of 1 m length at 10 cm apart on 7th July 2019. Twenty four days old seedlings were transplanted on prepared puddled field fertilized with N: P: K @ 120:40:30 kg ha^{-1} where half dose of nitrogen was applied as a basal dose and 1/4th at tillering and remaining dose at booting stage. For each genotype, single seedling was transplanted at 15 cm apart in two rows of 1.5 m length with 20 cm row-row difference in two replications. A weedicide Pendimethalin was sprayed @ 2 mL L^{-1} on next day of transplanting to inhibit weeds. An insecticide Chloropyriphos 50% EC + Cypermethrin 5% EC was sprayed @ 1 mL L^{-1} at tillering, booting and milking stage to reduce leaf defoliators/leaf rollers and other insects infestation.

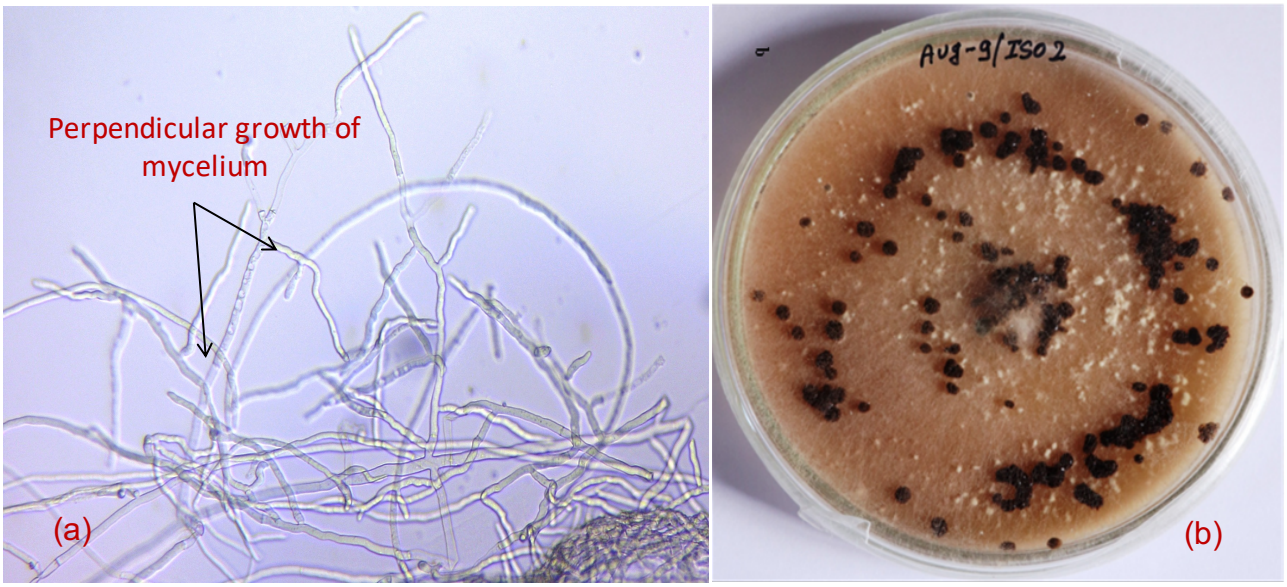

Figure 1. (a) = Mycelium of *R. solani* with perpendicular growth of hyphae, (b) =Pure culture of *R. solani* AG1-IA with brown/black color sclerotia used for inoculation

2.3 Pathogen and Inoculation

Leaves of rice with typical sheath blight symptoms were collected from a local farmer's field, disinfected with 75% ethanol and symptomatic portions were cut into 1 cm pieces. Four leaf pieces were placed on a Whatman filter paper wetted with sterile water, kept in 9 cm petri dish and incubated in a BOD (Biological oxygen demand) incubator at 25±1 ºC for 1 week. Single sclerotium of *R. solani* was transferred on 2% PDA media and incubated in BOD incubator at 25±1 ºC for 1 week. Seven days old sclerotia (Fig. 1b) with 0.2 mg weight were harvested and used for inoculation. Single tiller from five central hills, of similar growth stage from two rows of each genotypes were randomly selected and tagged with red wool. Single sclerotium covered with thin layer of cotton, wetted with sterile water to maintain humidity, was placed inside the sheath of second lower most leaf at late tillering to booting stage (Park et al., 2008).

2.4 Disease assessment

Four disease variables *viz.* percent diseased leaf incidence (PDLI), percent diseased tiller incidence (PDTI), percent relative collar height infection (PRCHI) and area under disease progress curve (AUDPC) were assessed. All four disease variables were assessed in the randomly selected inoculated and tagged tillers hill^{-1}. The PDLI was assessed by inspecting and counting the number of leaves with sheath blight lesions to total number of leaves in inoculated tillers at milk to dough growth stage and multiplying by 100 (Willocquet et al., 2011). The PDLI was measured to elucidate the degree of horizontal increment of the disease in the respective rice genotypes. Similarly PDTI was assessed by counting number of infected tillers to total number of tillers and multiplying by 100 at milking to dough stage of the rice genotypes. The PDTI was measured to understand the spreading ability of *R. solani* to corresponding genotypes under this experiment. The PRCHI was recorded by measuring the length of lesions on collar sheath (inoculated site) to total collar length at milk to dough growth stage and multiplying by 100. The PDTI was measured to correlate intensity of vertical increment of disease in rice genotypes. For estimation of AUDPC three recording for disease severity were performed *viz.* first after 10 days of inoculation, second and third at one week intervals. Disease severity was assessed by inspecting the proportion of lesions infection on inoculated tillers. AUDPC was estimated from disease severity metric by following the formula of (Shaner, 1977) as:

$$AUDPC = \sum_{i=0}^{n-1} [\{(Y_i + Y_{i+1})/2\} \times (t_{i+1} - t_i)] \quad (1)$$

where, Y_i = disease severity at time t_i, $(t_{i+1} - t_i)$ = Time (days) between two consecutive disease scores, n =number of dates at which rice sheath blight was recorded.

2.5 Statistical analysis

Data entry and processing was carried out using Microsoft Office Excel 2007. Analysis of variance (ANOVA), mean estimation and correlation analysis were done with the software- R studio, version

Figure 2. Response of rice genotypes to *R. solani* at late tillering stage. (a) highly susceptible showing sheath blight lesions covering all the portion of sheath collar and lower leaf in Sukkha Dhan 4, (b) susceptible showing sheath blight lesions partly covering the sheath collar and lower leaf), (c) moderately susceptible showing sheath blight lesions covering less than half of sheath collar and on adjacent leaf, and (d) moderately resistant showing sheath blight lesions restricted to inoculation site and not infecting the adjacent leaf in Hardinath-3

4.0.2 (2020) using package Agricolae version 1.3-3 (De Mendiburu, 2020).The statistical significance (alpha) was declared at 5% level of probability.

3 Results

The analysis of variance revealed that all the disease variables considered in this study *viz*. percent disease leaf incidence (PDLI), percent diseased tiller incidence (PDTI), percent relative collar height infection (PRCHI) and area under disease progress curve (AUDPC) were significant among genotypes (Table 1). On the basis of mean AUDPC value the genotypes were categorized as resistant (<200), moderately resistant (201-400), moderately susceptible (401-600), susceptible (601-800) and highly susceptible (>800). Among 42 genotypes Sabitri, GSR310 and Hardinath-3 were found moderately resistant with AUDPC values 217.99, 252.78 and 214.67 respectively (Table 2). Out of 42 genotypes, IR15 D110, Pant-1, NR 2152-23-1-2-1-1-1-1, IR 82635-B-B-114-3 were found moderately susceptible with mean AUDPC values 438.48, 445.55, 421.81 and 437.59 respectively (Table 2). Furthermore 33 genotypes were found susceptible whereas Pant-2 and Sukha dhan-4 showed highly susceptible response to sheath blight with mean AUDPC values 808.44 and 811.67 (Table 2).

Mean value range of PDLI, PDTI and PRCHI for moderately resistant genotypes was 30.98-31.67, 10.56-15 and 9.01-28.64 respectively (Table 2). Similarly mean value range of PDLI, PDTI and PRCHI for moderately susceptible rice genotype was 31.25-51.39, 25.82-38.75 and 22.18-45.8 respectively (Table 2). Furthermore mean value range of PDLI, PDTI and PRCHI for susceptible rice genotypes were 43.2-77.09, 35.36-100 and 35.63-95.46 respectively. Moreover mean value range of PDLI, PDTI and PRCHI for highly susceptible genotype was 56.67-57.09, 52.78-55.83 and 63.29-68.34 respectively (Table 2).

Correlation analysis revealed that PDLI was positively and significantly correlated with PDTI, PRCHI and AUDPC with correlation coefficient value 0.62, 0.47 and 0.75, respectively (Fig. 3). Similarly PDTI showed significant and positive correlation with PRCHI and AUDPC with correlation coefficient value 0.51 and 0.68 respectively (Fig. 3). Furthermore PRCHI also showed significant and positive correlation with AUDPC with correlation coefficient value 0.62 (Fig. 3).

4 Discussion

Screening of crop varieties against various crop diseases is necessary (Mew et al., 2004) and continuous process not only require for identifying the source of resistance genes or QTLs but also to identify the emergence of virulence pathotype against a particular crop disease (Singh et al., 2016; Wang et al., 2011). Among four variables studied in this experiment Area under disease progress curve was significant among genotypes. In this experiment we found moderately resistant rice genotypes *viz*. GSR 310, Sabitri and Hardinath with low AUDPC value 252.78, 217.99 and 214.67 per day, respectively. Moreover highly susceptible rice genotypes *viz*. Pant 2 and Sukha Dhan-4 had high AUDPC value 808.44 and 811.67 per day, respectively. Chaudhary (2016) also considered disease severity / AUDPC as one of the important variable for measuring sheath blight resistance in rice. He evaluated twelve rice genotypes and concluded resistant rice genotypes *viz*. Sabitri, Jasmine-85 and Betichikon having low disease severity. PDLI variable was studied to characterize the rice genotypes ability to facilitate the spread of sheath blight pathogen across the tillers and rice plants (Hashiba, 1984). This study revealed that 30-32% leaves were infected in moderately resistant rice genotypes *viz*. Sabitri, GSR310 and Hardinath-3 whereas 40-80% leaves were infected in susceptible genotypes NR 2175-66-2-3-1-140 and highly susceptible genotypes i.e. Pant 2 and Sukha Dhan-4. Similar disease variable i.e. lesion length and lesion number on tillers were studied by Willocquet et al. (2011) to quantify the components of resistance among rice varieties. They concluded that resistance rice varieties had the lowest lesion number (6.2 per tiller) with shortest lesion length whereas susceptible varieties had maximum lesion number (9.3 per tiller) and highest lesion length.

Furthermore disease variable, PDTI (percent diseased tiller incidence) measured the extensification of sheath blight disease. This experiment showed that PDTI value was less in moderately resistant genotypes *viz*.10.56% in GSR 310, 13.03% in Sabitri and 15% in Hardinath-3 whereas higher (35-100%) in susceptible/highly susceptible genotypes as 100% in IR 103588-77-1-2-3 genotype. PDTI explains whether the extensification of *R. solani* from tillers to tillers is dependent on genetic makeup of rice genotype. Willocquet et al. (2011) assessed 200 rice accessions for their susceptibility to sheath blight disease and found strong correlation between percent diseased tillers and sheath blight severity. Moreover in this studied percent relative collar height infection (PRCHI) was studied to discriminate the rice genotypes in terms of the rate of initiation of infection and expansion of lesion from inoculation site to upward and adjacent tillers. This study revealed that in moderately resistant rice genotypes PRCHI value was 9.01 for Hardinath-3, 21.54 for Sabitri and 28.64 for GSR 310 whereas in susceptible genotypes PRCHI value was 86.19 in NR2170-1-1-1-4-1-1-1 and 95.46 IR 2168-44-44-2-1-1-1-2-1-1. The genotypes Sabitri, Hardinath and GSR 310 had low PRCHI value which indicates slow growth and colonization of *R. solani* on these

Table 1. Analysis of variance for PDLI, PDTI, PRCHI and AUDPC

Source	DF	Mean square			
		PDLI	PDTI	PRCHI	AUDPC
Genotypes	41	201.0***	664.9***	749.8***	46051***
Replication	1	3.8	282.3	526.4	866
Residual	41	56.6	161.5	57.3	1867

** and *** significant at 0.05 and 0.001, respectively

Table 2. Least squares means with least significant difference for five traits measured in 42 rice genotypes

Sl. no	Pedigree	PDLI	PDTI	PRCHI	AUDPC	Host response
1	Pant-2	56.67	55.83	68.34	808.44	HS
2	TN-1	52.5	35.36	55.77	693.38	S
3	NR2181-5-1-1-6-1-1-1	57.36	45	56.44	761.96	S
4	IR 15D 110	41.67	26.93	28.89	438.48	MS
5	Pant-1	38.34	26.19	22.18	445.55	MS
6	Radha-4	56.67	65.21	59.21	743.3	S
7	NR 2180-20-2-5-1-1-1-1	48.89	58.34	58.81	665.84	S
8	NR 2152-23-1-2-1-1-1-1	31.25	25.82	25.87	421.81	MS
9	NR 2170-1-1-1-4-1-1-1	50.28	39.85	86.19	616.64	S
10	NR 2188-3-2-4-1-1	46.67	55	80.2	692.48	S
11	HHZ3-SAL1-Y1-Y1	59.59	44.85	35.63	793.35	S
12	NR 2170-150-5-3-2-1-1-1	47.5	42.68	74.57	732.43	S
13	NR 2175-66-2-3-1-1	77.09	52.28	51.65	745.97	S
14	NR 2179-82-2-4-1-1-1-1	43.2	46.25	75.26	685.81	S
15	IR 102885-31-11-4-11	59.59	56.94	55	640.37	S
16	IR 102885-2-74-17-2-3	53.34	49.2	65.53	640.42	S
17	NR 2157-144-1-3-1-1	59.45	57.59	56.82	783.89	S
18	IR 2168-44-2-1-1-1-2-1-1	46.67	70.24	95.46	646.55	S
19	NR 2157-122-1-2-1-1-1	55	46.43	66.44	622.63	S
20	2015 SA 4	54.45	73.34	43.93	668.27	S
21	2015 SA 22	51.39	46.43	62.59	758.73	S
22	IR 08L 201	56.11	50	40.11	692.26	S
23	Sukha Dhan-4	57.09	52.78	63.29	811.67	HS
24	Ghaiya-1	60	51.6	54.55	692.18	S
25	HHZ6-DT1-LT1-LT1	68.33	83.34	78.89	782.27	S
26	IR 86515-19-1-2-1-1-1-1	51.25	49.21	66.17	670.63	S
27	HHZ25-DT9-Y1-Y9	50	70	54.2	730.27	S
28	GSR 310	31.67	10.56	28.64	252.78	MR
29	IR 14L 363	60.28	71.67	47.62	688.28	S
30	IR 103588-77-1-2-3	54.59	100	50	727.24	S
31	IR 103575-76-1-1-B	51.11	38.42	64.11	750.33	S
32	Sabitri	30.98	13.03	21.54	217.99	MR
33	Hardinath-3	31.11	15	9.01	214.67	MR
34	IR 82589-B-B-95-2	52.78	57.78	51.46	669.26	S
35	IR 82635-B-B-114-3	51.39	38.75	45.8	437.59	MS
36	HHZ12-SAL2-Y3-Y2	58.61	60.72	90	611.03	S
37	IR 15L 1745	52.5	43.18	60.61	549.69	S
38	NR 2168-44-2-1-1-1-2-1-1	69.45	53.34	54.2	679.52	S
39	NR 2170-5-5-1-6-1-1-3-1	56.67	50.23	73.87	683.44	S
40	IR 13F 228	67.22	56.82	85.65	735.5	S
41	NR 2169-10-1-1-6-2-1-3-1	53.75	58.75	65.28	765.11	S
42	Radha-13	52.78	78.57	58.98	597.62	S
LSD		15.2	25.67	15.28	87.26	

Table 3. Mean value range of disease variables among rice genotypes manifested different response to sheath blight disease

Host response	PDLI	PDTI	PRCHI	AUDPC
Moderately resistant (MR)	30.98-31.67	10.56-15	9.01-28.64	214.67-252.78
Moderately Susceptible (MS)	31.25-51.39	25.82-38.75	22.18-45.8	421.81-445.55
Susceptible (S)	43.2-77.09	35.36-100	35.63-95.46	549.69-793.35
Highly susceptible (HS)	56.67-57.09	52.78-55.83	63.29-68.34	808.44-811.67

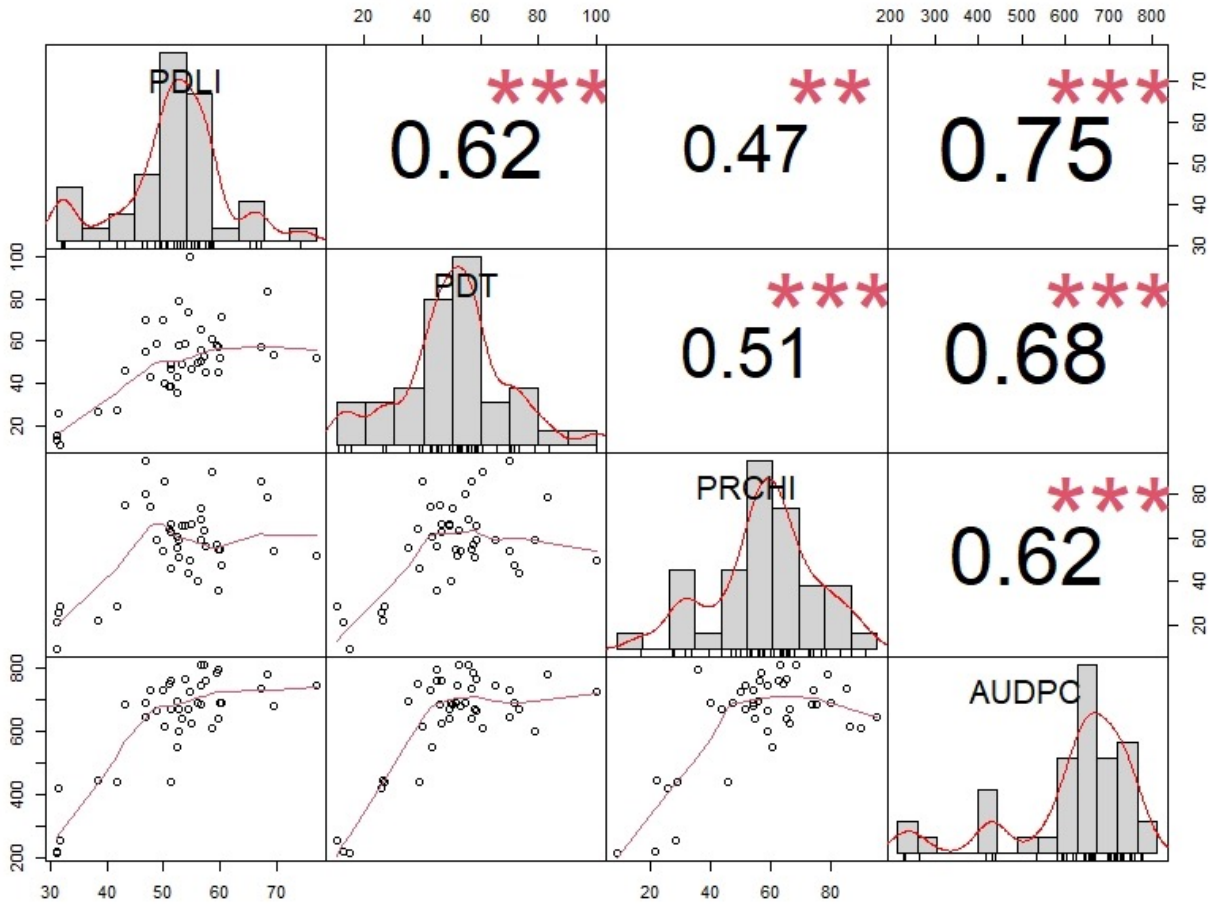

Figure 3. Correlation matrix showing relation between PDLI, PDTI, PRCHI and AUDPC value

rice genotypes. Pavani et al. (2020) had studied the relative lesion height for characterizing the 196 rice genotypes against sheath blight of rice and found 27 genotypes with relative lesion height below 20% and characterized as moderately resistant but we believe PRCHI should also be considered as it describes the interaction of rice genotypes versus vertical progress of disease.

The selection of resistant cultivars is the most economical and environmentally beneficial means of reducing losses caused by sheath blight of rice. Cultural control methods (Peters et al., 2001; Rush and Lee, 1992) are insufficient and the use of fungicides (Groth, 2005) may not be economically or environmentally sustainable. Transformation of rice cultivars with defense genes has exhibited only partial resistance against sheath blight of rice (Kalpana et al., 2006). A concerted effort is currently underway in the United States to identify QTL for sheath blight resistance and to study the key genes and underlying mechanisms of the sheath blight resistance. To resolve these issues, uniform and effective inoculation and precise evaluation methods are required for detailed genetic, molecular, biochemical, and functional genomics analyses and for measuring quantitative differences in sheath blight resistance among rice breeding lines, mutants, and transgenic plants.

5 Conclusion

This study emphasized resistance components *viz*. PDLI (Percent diseased leaf incidence), PDTI (Percent diseased tiller incidence), PRCHI (Percent relative collar height infection), and AUDPC (Area under disease progress curve) for evaluation of advanced rice genotypes against sheath blight of rice. This study revealed three genotypes *viz*. Sabitri, GSR-310 and Hardinath-3 as moderately resistant with mean AUDPC values 217.99, 252.78 and 214.67 per day respectively among forty two rice genotypes. These genotypes could be could be used for QTL analysis/ as donor parents in breeding program/ evaluated for yield potential and processed for variety release. Also these moderately resistant rice genotypes could be used for QTL analysis and as donor parents in breeding program. Furthermore IR 15D 110, Pant-1, NR 2152-23-1-2-1-1-1-1 and IR 82635-B-B-114-3 were found moderately susceptible with mean AUDPC values 438.48, 445.55, 421.81 and 437.59 respectively. These moderately susceptible rice genotypes could also be further evaluated and if found with high yield potential could be processed for variety release.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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